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(71) Applicant: MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

(72) Inventors: BEATY, Julie, A.; 2259 Florissant, #11, Florissant, MO 63031 (US). COOPER, Steven, R.; 2020 LaChelle, Maryland Heights, MO 63146 (US). McLOUGHLIN, Margaret, A.; 16 Bon Hills, St. Louis, MO 63132 (US).

(74) Agents: STIERWALT, Brian, K. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

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(54) Title: STABILIZATION OF VOLTAGE SENSITIVE DYES

(57) Abstract

The invention relates to stable aqueous solutions of voltage sensitive dyes. Particularly the invention relates to the proper formulation and storage conditions that provide marketable aqueous solutions of voltage sensitive dyes for use as optical imaging contrast agents.

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STABILIZATION OF VOLTAGE SENSITIVE DYES

FIELD OF THE INVENTION

The invention relates to voltage sensitive dyes. More particularly, the invention relates to storage stable compositions comprising voltage sensitive dyes.

BACKGROUND

Experiments with voltage-sensitive dyes have been calculated to optically image neural activity (Optical Imaging of Neuronal Activity Grinvald, A.; Frostig, E.L.; Hildesheim, R. Physiological Reviews 1988, 68, 1285-1366; Real-time Imaging of Evoked Activity in Local Circuits of the Salamander Olfactory Bulb, Kauer, J.S. Nature 1988, 331, 166-168; Voltage-sensitive dyes reveal a modular organization in monkey striate cortex, Blasdel, G.G.; Salama, G. Nature 1986, 321, 579-585). These experiments surgically exposed and bathed the tissues of interest in solutions of the dyes. Changes in the membrane potentials of the tissues activate changes in the absorption or fluorescence of the voltage-sensitive dyes. It has been reported that changes in intracranial light absorbance through the intact skull can be detected when induced by a bolus of indocyanine green (Intracerebral penetration of Infrared light, McCormick, P.W.; Stewart, M.; Lewis, G.; Dujovny,

M.; Ausman, J.I. J. Neurosurg 1992, 76, 315-318).

Indocyanine green, for example, is a voltage sensitive dye with an absorption maxima around 805 nm (the isosbestic point of the hemoglobin/deoxyhemoglobin system). This absorption makes it possible to monitor blood concentrations by ear densitometry. Indocyanine green has been used for determining cardiac output, hepatic function and liver blood flow. It also has been used for plasma volume measurement and for regional angiography of organs including the eyes, kidneys and lungs (Gennaro, A. R., Ed Remington's Pharmaceutical Sciences Easton, PA: Mack Publishing Company, 1990, 1279. Indocyanine green, in vivo, is readily bound by plasma proteins and remains in the blood stream through one circulation of the heart and lungs. It is then transported to the bile and excreted to the small intestine, without readsorbtion. Protein binding of indocyanine green prevents both extravascular distribution and metabolism. Indocyanine green is removed from the plasma almost exclusively by hepatic function (Osol, A.; Pratt, R. The United States Dispensatory Philadelphia, Toronto: J. B. Lippincott Company, 1973, 615).

Although numerous applications for the use of voltage sensitive dyes are available and evolving, most of these dyes are difficult to store. Aqueous solutions of indocyanine green, for example, rapidly decompose when irradiated with incandescent light (e.g., T1/2 19h in deionized water) (Indocyanine green: pharmokinetics in the rabbit and relevant studies of its stability and purity, Heintz, R.; Svensson, C.K.; Stoeckel, K.; Powers, G. J.; Lalka, D. J. Pharm. Sci. 1986, 75, 398-

402). Room temperature solutions of indocyanine green in methanol or bile remain stable (T1/2 > 1y), but indocyanine green in duodenal fluid or distilled water decompose rapidly (e.g., T1/2 3.6d and T1/2 3.6d and T1/2 1.4 d, respectively) (Physiocochemical studies of indocyanine green (ICG): abscorbance/concentration relationship, pH tolerance and assay precision in various solvents, Bjoernsson, O.G.; Murphy, R.; Chadwick, V.S. Experientia 1982, 38, 1441-1442). Plasma proteins (e.g., human serum albumin) inhibit decomposition from incandescent light (Light-absorbing properties, stability and spectral stabilization of indocyanine green, Landsman, M.L.J.; Kwant, G.; Mook, G.A.; Zijlstra, W.G. J. Appl. Physiol. 1976, 40, 575-583) as does protection from light (Studies on the stability of indocyanine green in serums, Nimata, H.; Yoshida, S.; Shimizu, N.; Yoneya, M.; Nishibe, M.; Matsubara, R. Rinsho Kensa 1974, 18, 320-322). The molecules lack obvious sources of instability and surprisingly, the extant literature (Stability studies on indocyanine green dye. Gathje, J.; Steuer, R.R.; Nicholes, K.R.K. J. Appl. Phisiol. 1970, 29, 181-185) does not address the role of oxygen in decomposition.

A need continues to exist for storage stable compositions of voltage sensitive dyes.

SUMMARY OF THE INVENTION

The invention relates to stable aqueous solutions of voltage sensitive dyes. Particularly the invention relates to the proper formulation and storage conditions that provide marketable aqueous solutions of voltage sensitive dyes for use as optical imaging contrast agents.

The invention is advantageous in allowing shipment and storage of the disclosed compositions either alone or in pre-filled syringes. Prior to this invention, on site preparation was necessary due to the poor stability of the solutions. In addition, prior to the invention, shipment and storage of such dyes occurred only with solid forms of the dyes.

The following definition of terms are set forth as used in this document. Voltage sensitive dyes refers to those compositions that reflect a change in their fluorescence or absorbance with changes in voltage. Storage stable refers to a composition of the invention that decomposes at a slower rate than a voltage sensitive dye without an air protecting reagent. A decomposition inhibiting amount refers to that amount that renders the aqueous solutions of the invention storage stable. Air protecting reagents refers to any composition capable of inhibiting decomposition of the voltage sensitive dyes due to air.

Detailed Description of Invention

It has been discovered that compositions of the invention have longer storage life (i.e., less decomposition) due to protection from light, and air, either separately, or in combinaticon. Some of the advantages of aqueous solutions of voltage sensitive dyes include the speed with which they can be used (i.e., no wait for solids to dissolve), the convenience with which they can be used (i.e., no worry about preparation for solution and whether or not all of the solids dissolved), the safety with which they can be used (i.e., less chance of microbial and particulate contamination), and the ease with which they can be used (i.e., less chance of getting any particulates from the stopper into the solution because the needle would only be pushed through once as opposed to twice for a reconstituted kit; no chance for similar contamination if a prefilled syringe is uses).

Voltage sensitive dyes for use in the present invention include Evans Blue (Aldrich) ($C_{34}H_{24}N_6Na_4O_4S_4$), Indocayanine green, merocyanine, cyanine dye, merocyanine dye (Aldrich), oxonol, oxonol dye, styryl, rhodanine merocyanine, indigo carmine ($C_{16}H_8N_2Na_2O_8S_2$) (Aldrich), sulphan (or Patent) Blue (Aldrich) ($C_{27}H_{31}N_2NaO_6S_2$) (Aldrich), congo red ($C_{32}H_{22}N_6Na_2O_6S_2$) (Aldrich), and fluorescein sodium ($C_{20}H_{10}NaO_5$) (Aldrich).

Suitable light protection measures to be used with compositions of the invention include the use of wrappings such as opaque wrappings, cardboard boxes, and styrofoam containers. It is especially advantageous to protect compositions of the invention from light that is the same as that absorbed by the compositions.

Suitable air protection measures to be used with compositions of the invention include the use of air protecting reagents. Examples of air protecting reagent are antioxidants, gases, and surfactants.

Suitable air protecting reagents for use with the invention include gases such as argon and nitrogen. The use of gases involves purging a vial containing solid dye with argon or nitrogen and sealing. Then bubble a buffered solution, which contains the desired antioxidant or surfactant in the appropriate proportion, with argon or nitrogen. Add the degassed solution to the solid dye and transfer the solution to a purged vial, or syringe. A stopper will not resist air indefinitely so a sealed ampule may be preferable.

Suitable antioxidants for use in practicing the invention include sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, acetone sodium metabisulfite, isoascorbic acid, thioglycerol, ascorbate, thiosorbitol, cysteine hydrochloride, sulfur dioxide, acetylcysteine, thiolactic acid, dithyiothreitol and glutathione (all available from Aldrich, Fisher and/or Fluka).

Surfactants and compositions that exhibit surfactant-like properties suitable for use with the invention include tweens, polysorbates (ICI or Sigma), Pluronics (ICI or Sigma), Polyethylene glycols (Sigma), and sodium carboxymethyl celluloses (Sigma). Surfactant and compositions with surfactant-like capabilities are believed to protect from air by associating with the

organic functionalities or incorporating the organic molecule into a micelle.

The amount of air protecting reagents for use with the invention range from about 0.01% to about 1.0% of an aqueous solution. Preferably a range from about 0.1% to about 0.25% is used. The amount of air protecting reagent generally depends upon the solubility of the particular air protecting reagent in aqueous solution.

For practical use it is beneficial that the storage stable aqueous solution of the invention be stable for at least the time period from the time of preparation (e.g., laboratory or pharmacy) until administration or use. Typicallly this period is from about a few minutes to about a few hours. Preferably the storage stability is from about a full day (24 hours) to about a few weeks. Most preferably the storage stability is for about a year (12 months) or longer.

A major advantage of voltage sensitive dyes is that time resolution is better than 1 millisecond (ms) compared to time resolution on the order of seconds for the commonly used intrinsic light signals. Thus, the invention is suitable for imaging in patients, typically warm blooded animals. A method for imaging using compositions of the invention comprises administering an imaging effective amount of a composition of the invention to a patient and then subjecting the patient to the desired imaging modality.

Examples of preferred voltage sensitive dyes for use with the invention include Evan's Blue, Indocyanine Green, Congo Red, Fluorescein Sodium, Sulphan Blue, and

Indigo Carmine. Examples of preferred air protecting reagents for use with the invention include Sodium Ascorbate, Glutathione, Dithiothreitol, Sodium Ascorbate, EDTA, Polysorbate 80, and Carboxymethylcellulose.

Other agents that may be added to the compositions of the invention comprising voltage sensitive dyes include those compositions for formulating diagnostic compositions. Such diagnostic compositions can be for enteral or parenteral administration and may include pharmaceutically acceptable buffers, electrolytes, surfactants, thixotropic agents, and the like. The diagnostic composition is administered in an imaging effective amount, an imaging effective amount being that amount necessary for obtaining the desired image. Diagnostic compositions contain an effective amount of the compositions of the invention along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageolusly contain a sterile aqueous solution or suspension of from about 0.05 to 1.0M of a composition according to the invention. Preferred parenteral formulations have a concentration of about 0.1M to about 0.5M. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations are liquids which include an effective amount of a composition of the invention in aqueous solution or

suspension. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the image. Such doses may vary, depending upon the particular composition employed, the organs or tissues which are the subject of the imaging procedure, the imaging procedure, the imaging equipment being used, and so forth. In general, parenteral dosages will range from about 0.01 to about 1.0MMol of composition of the invention per kg of patient body weight. Preferred parenteral dosages range from about 0.05 to about 0.5MMol per kg of patient body weight. Enteral dosages generally range from about 0.5 to about 100 MMol, preferably from about 1.0 to about 10 MMol per kg of patient body weight.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blodded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure.

The following examples illustrate the specific embodiments of the invention described in this document. As would be apparant to skilled artisans, various changes and modifications are possible and are contemplated within the scope of the invention described.

EXAMPLES

The examples set forth show that protection from light and air inhibit decomposition of voltage sensitive dyes.

A series of experiments were carried out on aqueous .006 mM solutions of indocyanine green. In each experiment decomposition was monitored by decreases in the maximal absorbance in the UV visible spectra between 600-900 nm. Initial experiments were done in duplicate using one air free solution and one air containing solution to help examine the effect of oxygen on decomposition; exposure to incandescent light caused both solutions to decompose; the oxygen-free solution, however, decomposed at a slightly lower rate. At this point many variables were investigated such as temperature, light source, and pH. In all cases the air free solution decomposed at a slower rate. In fluorescent light, decomposition was reduced considerably for both solutions. Almost no decomposition was observed when the solutions were heated in the absence of light, but heat did appear to accelerate decomposition in the presence of incandescent light. Lowering the pH from 9.45 to 7.39 had no affect on decompsition. EDTA was added to the solutions to eliminate free metal ions, but there was no observable consequence. Ascorbate was added to an air free buffered (pH 7.39) solution that was exposed to incandescent light and decomposition was inhibited.

Materials preparations

Indocyanine green (ICG), sodium bisulfite, L-ascorbic acid, and the $Na_2H_2PO_4$ were purchased from Aldrich. The $NaH_2PO_4H_2O$, and the dicalcium ethylenediaminetetraacetic acid (EDTA) were obtained from Mallinckrodt. Solutions were prepared in 250 mL volumetric flasks and transferred into 1.0 cm cuvettes with lids for the absorbance measurements. The solutions were left in the cuvettes for the duration of the experiment. The solutions were irradiated with incandescent light using a desk lamp containing a 60 W bulb; the light was placed at a distance of approximately 5 cm from the cuvettes. Heating of the solutions was accomplished via a hot water bath maintained at a temperature between 50-53C. UV-Visible spectra were recorded on a Cary 3E spectrophotometer over a region of 600 to 900 nm. The phosphate buffer was prepared by dissolving 0.2975 g of $NaH_2PO_4H_2O$ and 1.0813 g of Na_2HPO_4 in 250 mL of deionized water. The pH of the buffer was 7.39.

Example 1:

1.25 mg of ICG was dissolved in 250 mL of deionized water. One aliquot of this solution was placed in a cuvette and degassed; a second aliquot was not degassed. Both cuvettes were irradiated as described above. (It should be noted, that irradiation does cause some warming of the solutions.) After 60 min., the solution without air decreased in absorbance from ~1.05 to 0.2; the solution with air decreased from 1.05 to 0.15. Thus under these conditions, the lack of oxygen has little effect on the rate of decomposition.

Example 2:

Analogous solutions to those in Example 1 were used, except this time the cuvettes were placed in a water bath to which ice was added as needed to prevent the temperature of the solution from rising above 30C. The solution without air showed a decrease in absorbance from 1.05 to 0.7 after 60 min.; the solution with air showed a decrease from 1.6 to 0.8. Although these solutions still display a slower decomposition than the uncooled ones above, it is unclear whether the decomposition is due to heat or light or both.

Example 3:

Analogous solutions to those in Example 1 were used, however they were not irradiated. Instead, the solutions were heated in a hot water bath at 50-53C. In the air free solution, the absorbance decreased from ~1.1 to 1.05 after 90 min.; the solution with air showed a decrease in absorbance from 1.6 to 1.3 over the same time frame. Comparing these results to those in Example 2 indicates that it is light, not heat, that is the major factor affecting the rate of decomposition of the dye.

Example 4:

Solutions analogous to those above were used once again. The samples were allowed to sit opened to the fluorescent ceiling lighting to see if such lighting would show the same effect as incandescent lighting. The absorbance of the degassed solution did not change significantly after 60 minutes and there was only a very

minor change in the air containing solution. After sitting overnight, the degassed solution had a decrease in absorbance from ~1.0 to ~0.4 and the air-conditioning solution had a decrease from ~1.0 to 0.05. The retardation in the decomposition with fluorescent light may be due to the fact that fluorescent light is more blue whereas incandescent light is more red and thus absorbs in the same region as the ICG.

Example 5:

1.0 mg of ICG was dissolved in 250 mL of phosphate buffer (described above). One aliquot of this solution was placed in a cuvette and degassed; a second aliquot was not degassed. Both cuvettes were irradiated with incandescent light. NOTE: from here on out, the term "light" refers to incandescent light. After 60 minutes, both solutions decreased in absorbance from ~1.0 to 0.2. Apparently, the presence of buffer causes the solutions to decompose at the same rate.

Example 6:

A solution analogous to that used in Example 5 was used, except this time, a spatula tip of solid sodium ascorbate was added also. After 60 min., the absorbance of an air-containing solution decreased from 1.0 to 0.7.

Example 7:

The same solution as in Example 5 was prepared, however 3 mL of a 0.0002 M solution of sodium ascorbate (4% of ICG concentration) was added, also. After 60 minutes, a

degassed solution showed a decrease in absorbance from 1.0 to 0.15. Comparing this to the result in Example 5, the presence of 0.0002 M ascorbate does not retard the decomposition of the ICG.

Example 8:

2.50 mg of ICG were dissolved in 250 mL of phosphate buffer. 1.5 mL of this solution was combined with 1.5 mL of 0.10 M ascorbate solution and irradiated. After 60 minutes, the absorbance of a degassed solution decreased from 0.3 to 0.2; a cooled solution (as described in Example 2) displayed a decrease in absorbance from 0.4 to 0.2. These results indicate that at sufficient concentration, ascorbate inhibits the decomposition of ICG.

Example 9:

A heaping scoop of sodium bisulfite was added to 3 mL of a solution containing 1.25 mg of ICG in 250 mL of phosphate buffer. In a degassed solution, the initial absorbance is ~0.3, however, after only 15 minutes, the absorbance is 0.0. Apparently, the bisulfite is destroying the dye.

Example 10:

2.50 mg of ICG were dissolved in 250 mL of buffer. 1.5 mL of this solution was combined with 1.5 mL of a 0.10 M

bisulfite solution. The initial absorbance of a degassed solution was 1.0, however it rapidly drops to ~0.5 and remains there up to 75 minutes. This result offers further support that the bisulfite is decomposing the ICG; this is also evident in that the light green colored ICG solution immediately becomes colorless upon adding the bisulfite. Thus, sodium bisulfite is not effective at inhibiting the decomposition of ICG.

__Example 11:__

1.0 mg of ICG was dissolved in 250 mL of phosphate buffer. 5.29 mL of 0.00014 M EDTA (4% of dye concentration) was added to this solution. After 60 min., a degassed solution showed a decrease in absorbance from 1.0 to ~0.1. Evidently, EDTA has no effect on slowing the rate of decomposition.

__Example 12:__

1.0 mg of ICG was dissolved in 250 mL of phosphate buffer. To this solution was added a spatula tip amount of solid EDTA. In an air-containing solution, the absorbance decreased from 0.9 to 0.25 after 60 minutes, thus, still no effect on slowing decomposition.

__Example 13:__

1.25 mg of ICG were dissolved in 250 mL of phosphate buffer. 3.0 mL of this solution was combined with 154 mL of a 0.0136 M EDTA solution. The absorbance of an air free solution decreased from 0.24 to 0.12 after 60

minutes; the absorbance of a cooled solution (as described in Example 2) decreased from ~0.4 to ~0.1 after 60 minutes. Even with an extreme excess of EDTA, the rate of decomposition of ICG is not retarded.

Example 14:

1.25 mg of ICG was dissolved in 250 mL of buffer. The initial absorbances measured 0.9 (degassed) and 0.8 (with air). The solutions were covered with foil and placed in the dark (in a cabinet). After 24 hours, the absorbances were unchanged. After 1 month, the absorbance readings were ~0.35 and below zero, respectively. Therefore, keeping the solutions in the dark slow the decomposition significantly, however it does not halt it completely.

Although the invention has been described with respect to specific modifications, the details thereof are not to be construed as limitations, for it will be apparent that various equivalents, changes and modifications may be resorted to without departing from the spirit and scope thereof, and it is understood that such equivalent embodiments are to be included therein.

CLAIMS

What is claimed is:

1. A storage stable aqueous solution comprising a voltage sensitive dye.

2. The composition of Claim 1 in which the dye is selected from the group consisting Evan's Blue, Indocyanine Green, Congo Red, Fluorecein Sodium, Sulfan Blue and Indigo Carmine.

3. The composition of Claim 2 in which the dye is Indocyanine Green.

4. The composition of Claim 1 in which the storage stable aqueous solution comprises a decomposition inhibiting amount of air protecting reagent.

5. The composition of Claim 4 in which the air protecting reagent is an antioxidant.

6. The composition of Claim 5 in which the antioxidant is selected from the group consisting of ascorbate, glutathione and dithiothreitol.

7. The composition of Claim 6 in which the antioxidant is ascorbate.

8. The composition of Claim 4 in which the air protecting reagent is a surfactant.

9. The composition of Claim 8 in which the surfactant is selected from group consisting of polysorbates, carboxymethylcelluloses and pluronics.

10. The composition of Claim 9 in which the surfactant is a polysorbate.

11. The composition of Claim 2 which comprises an antioxidant selected from the group consisting of ascorbate, glutathione and dithiothreitol.

12. The composition of Claim 9 in which the dye is selected from the group consisting of Evan's Blue, Indocyanine Green, Congo Red, Fluorecein Sodium, Sulfan Blue and Indigo Carmine.

13. The composition of Claim 11 in which the dye is Indocyanine Green and the antioxidant is ascorbate.

14. The composition of Claim 12 in which the dye is Indocyanine Green and the surfactant is a polysorbate.

15. A method for making an aqueous solution of a storage stable voltage sensitive dye which comprises formulating the dye with a decomposition inhibiting amount of an air protecting reagent.

16. The method of Claim 15 in which the air protecting reagent is an antioxidant.

17. The method of Claim 16 in which the antioxidant is selected from the group consisting of ascorbate, glutathione and dithiothreitol.

18. The method of Claim 17 in which the antioxidant is ascorbate.

19. The method of Claim 15 in which the dye is selected from the group consisting of Evan's Blue, Indocyanine Green, Congo Red, Fluorecein Sodium, Sulfan Blue and Indigo Carmine.

20. The method of Claim 9 in which the dye is Indocyanine Green.

21. The method of Claim 19 in which the dye is Evan's Blue.

22. The method of Claim 16 in which the dye is selecled from the group consisting of Evan's Blue, Indocyanine Green, Congo Red, Fluorecein Sodium, Sulfan Blue and Indigo Carmine.

23. The method of Claim 22 in which the dye is Indocyanine Green.

24. A method of using a storage stable aqueious solution comprising a voltage sensitive dye which comprises administering an imaging enhancing amount of the dye to a patient and

illuminating the dye.

25. The method of Claim 24 in which the dye is selected from the group consisting of Evan's Blue, Indocyanine Green, Congo Red, Fluorescein Sodium, Sulphur Blue and Indigo Carmine.

26. The method of Claim 25 in which the dye is Indocyanine Green.

27. The method of Claim 25 in which the dye is Congo Red.

28. The method of Clairn 24 in which the storage stable aqueous solution comprises a decomposition inhibiting amount of an antioxidant.

29. The method of Claim 28 in which the antioxidant is selected from the group consisting of ascorbate, glutathione and dithiothreitol.

30. The method of Claim 29 in which the antioxidant is ascorbate.

31. The method of Claim 24 in which the storage stable aqueous solution comprises a decomposition inhibiting amount of a surfactant.

32. The method of Claim 31 in which the surfactant is selected from the group consisting of

polysorbates, carboxymethylcellulose and pluronics.

33. The method of Claim 32 in which the surfactant is polysorbate.

34. The method of Claim 29 in which the storage stable aqueous solution comprises the dye Indocyanine Green.

35. The method of Claim 32 in which the solution comprises the dye Indocyanine Green.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04267

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61B 5/00; C09B 69/00; C09K 11/06
US CL : 8/444,658; 128/654,665; 252/301.16, 301.17, 301.36
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 8/444,658; 128/654,665, 252/301.16, 301.17, 301.36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,526,701 (Rubin) 02 July 1985, Example 1(a).	1-2, 4-8, 15-19, 22
X	Journal of Applied Physiology, Vol. 40, No. 4, issued April 1976 (USA), M.L.J. Landsman et al., "Light-Absorbing Properties, Stability, and Spectral Stabilization of Indocyanine Green" see pages 575-582, especially page 582.	1-3
X	Journal of Applied Physiology, Vol. 29, No. 2, issued August 1970 (USA), J. Gathje et al., "Stability Studies on Indocyanine Green Dye" see pages 181-185, especially page 181.	1-3, 24-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

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PAUL LIEBERMAN
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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Pharmaceutical Sciences, Vol. 75, No. 4, issued April 1986, R. Heintz et al., "Indocyanine Green: Pharmacokinetics in the Rabbit and Relevant Studies of Its Stability and Purity" see pages 398-402, especially page 398.	1-3, 24-26
Y	US, A, 4,369,250 (Gindler) 18 January 1983, col. 6, line 31-col. 7, line 4.	1-23
Y	US, A, 4,478,818 (Shell et al.) 23 October 1984, col. 10, lines 35-65.	5-7, 11, 13, 16-18, 22-23
P, Y	US, A, 5,266,302 (Peyman et al.) 30 November 1993, col. 1, lines 13-20.	24-26

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PCT/US94/04267

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, DIALOG
search terms: voltage, indocyanine green, ascorbate, glutathione, dithiothreitol, polysorbate, carboxymethylcellulose, pluronics, Evan's blue, congo red, fluorescein sodium, sulfan blue, indigo carmine